

# Population pharmacokinetic modelling of total and unbound cefazolin plasma concentrations as a guide for dosing in preterm and term neonates

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**Objectives:** Cefazolin is frequently administered for antimicrobial prophylaxis and treatment of infections. In neonates, pharmacokinetic observations are limited and dosing regimens variable. The aim of this study was to describe the pharmacokinetics of cefazolin in neonates based on total and unbound concentrations to optimize cefazolin dosing.

**Methods:** Thirty-six neonates [median birth body weight 2720 (range 540–4200) g, current body weight (cBW) 2755 (830–4200) g and postnatal age (PNA) 9 (1–30) days] receiving intravenous cefazolin (50 mg/kg/8 h) were included. Based on 119 total and unbound plasma concentrations, a population pharmacokinetic analysis with a covariate analysis was performed. Monte Carlo simulations were performed aiming for unbound concentrations above an MIC of 8 mg/L (>60% of the time) in all patients.

**Results:** A one-compartment pharmacokinetic model was developed in which total and unbound concentrations were linked by maximum protein binding ( $B_{\max}$ ) of 136 mg/L and a dissociation constant ( $K_D$ ) for cefazolin protein binding of 46.5 mg/L. cBW was identified as covariate for volume of distribution ( $V$ ), bBW and PNA for clearance and albumin plasma concentration for  $B_{\max}$ , explaining 50%, 58% and 41% of inter-individual variability in  $V$ , clearance and  $B_{\max}$ , respectively. Based on Monte Carlo simulations, a body weight- and PNA-adapted dosing regimen that resulted in similar exposure across different weight and age groups was proposed.

**Conclusions:** A neonatal pharmacokinetic model taking into account total and unbound cefazolin concentrations with saturable plasma protein binding was identified. As cBW and PNA were the most important covariates, these may be used for individualized dosing in neonates.

**Keywords:** developmental pharmacology, antibiotics, protein binding

## Introduction

Based on a European survey, 15% of antimicrobial use for surgical prophylaxis in children is accounted for by first-generation cephalosporins.<sup>1</sup> In a US point prevalence survey of patients in paediatric intensive care units and neonatal intensive care units, cefazolin was used in 17.6% and 1.2% of patients on the day of the survey, respectively.<sup>2</sup> Indications for cefazolin administration in neonates are mainly prophylactic (72%), and to a lesser extent therapeutic (17%) (e.g. coagulase-negative staphylococcal sepsis)<sup>3</sup> or empirical (11%).<sup>2</sup> While the pharmacokinetics of cefazolin have been described in adults, information on cefazolin pharmacokinetics

in early life is limited.<sup>4–6</sup> Cefazolin is highly bound to human serum albumin and this binding displays saturation.<sup>7–9</sup> Only the unbound cefazolin distributes to the extravascular compartments and undergoes renal elimination. Neonates have a proportionally large total body water volume, immature renal function and low albumin level.<sup>10–12</sup> This population-specific physiology probably affects cefazolin disposition.

Efficacy of cefazolin relates to the time unbound cefazolin concentrations exceed the MIC for a given pathogen ( $T_{>MIC}$ ).<sup>13</sup> In neonates, often regarded as vulnerable and even immunocompromised patients, effective cefazolin therapy requires at least 60% of  $T_{>MIC}$ .<sup>14</sup>

Up to now, the neonatal clearance (CL) values for cefazolin described in the literature are based on total cefazolin concentrations only, necessitating a cefazolin pharmacokinetic analysis integrating both total and unbound drug concentrations in neonates. Moreover, currently used cefazolin dosing regimens for neonates are variable (Table S1, available as Supplementary data at JAC Online).<sup>15–21</sup>

Therefore, the aim of this study was to describe the pharmacokinetics of cefazolin in preterm and term neonates on the basis of both total and unbound cefazolin concentrations. Based on the final pharmacokinetic model, Monte Carlo simulations were performed to illustrate exposure to cefazolin in (pre)term neonates following currently used dosing regimens. Subsequently, a model-based dosing regimen was developed for preterm and term neonates.

Methods

Ethics, study population and drug dosing

Inclusion of patients in this study was based on a previously published cohort of 39 neonates and young infants, all admitted to the Neonatal Intensive Care Unit of the University Hospitals Leuven Belgium.<sup>8</sup> The study was approved by the ethics board of the hospital and registered at ClinicalTrials.gov (NCT01295606), and parental written informed consent was obtained. Inclusion was feasible if cefazolin (Cefazolin Sandoz®, Sandoz, Vilvoorde, Belgium) was administered intravenously as routine surgical prophylaxis. At induction of surgery, a cefazolin dose of 50 mg/kg was administered over 30 min. According to the local standard of care (depending on foreign body implantation or contamination risk of the procedure), additional cefazolin dose(s) of 50 mg/kg could be administered every 8 h up to a maximum of 48 h. As in the present analysis only neonates with postnatal age (PNA) 1–30 days were included, three patients (PNA 48, 51 and 108 days) were excluded from the original dataset.<sup>8</sup> Clinical characteristics were extracted from the medical files. Albuminaemia (g/L), indirect serum bilirubin concentrations (mg/dL) and serum creatinine (mg/dL) registered in a time interval of 24 h before or after the first cefazolin administration were collected. Plasma free fatty acid concentrations were determined in samples at the end of the study. Clinical characteristics of the study population are presented in Table 1.

Blood sampling

Blood samples were collected in lithium–heparin tubes at fixed timepoints of 0.5, 2, 4 and 8 h after the first cefazolin administration and subsequently at 8 h intervals prior to each scheduled cefazolin administration,

to determine total and unbound cefazolin concentrations. However, the number of samples collected from each patient was limited since the pre-defined total volume of blood available for sampling per patient was maximized to 1 mL/kg body weight. Blood samples (0.6 mL/sample) were immediately centrifuged (5 min, 4500 rpm at 4°C) and the resulting 0.3 mL of plasma was stored at –20°C in two aliquots of 0.15 mL.

Drug assay

Total and unbound cefazolin concentrations were determined by HPLC after solid-phase column extraction. The initial method was developed in our laboratory<sup>22</sup> and adapted for measurement of cefazolin in small-volume plasma samples.<sup>8</sup> The lower limit of quantification for cefazolin was 0.1 mg/L, with a coefficient of variation <20%. Intra-assay precision and accuracy averaged 3.9% and 5.5%, respectively. Inter-assay precision and accuracy averaged 5.7% and 6.8%, respectively, which is in line with FDA analytical recommendations.<sup>23,24</sup>

Biochemical assays

Albumin, indirect bilirubin and creatinine (enzymatic) were quantified with a Roche Modular P analyser (Roche Diagnostics, Basel, Switzerland). Free fatty acids were determined with a kit from DiaSys Diagnostic Systems (Holzheim, Germany).

Population pharmacokinetic analysis

Model development

The population pharmacokinetic analysis was performed using the non-linear mixed-effect modelling software NONMEM version 6.2 (Globomax LLC, Hanover, MD, USA) using the first-order conditional estimation method with the interaction option (FOCE-I). Tools used to visualize and evaluate the model were S-Plus version 6.2.1 (Insightful, Seattle, WA, USA) with NM.SP.interface version 05.03.01 (© LAP&P Consultants BV, Leiden, The Netherlands), PsN and R (version 2.10.1).

The model-building process was performed in a stepwise manner: (i) choice of the structural model; (ii) choice of the statistical sub-model; (iii) choice of the covariate model; and (iv) model evaluation. Different diagnostic tools were used to discriminate between the different models.<sup>25</sup> A decrease in objective function value (OFV) of ≥3.9 points was considered statistically significant (*P*<0.05 based on the χ<sup>2</sup> distribution, for nested models). Furthermore, the goodness-of-fit plots were evaluated. Finally, the total number of parameters, visual improvement of individual plots, correlation matrix, CIs of parameter estimates, ill conditioning<sup>26</sup> and shrinkage<sup>27</sup> were assessed.

Structural and statistical sub-model

One- and two-compartment pharmacokinetic models were fitted to both total and unbound cefazolin concentrations using NONMEM VI, subroutine ADVAN6, TOL = 3. Unbound cefazolin concentrations were related to total cefazolin concentrations by the following equation, taking into account non-linear protein binding:<sup>28</sup>

$$C_{unbound} = \frac{1}{2} \times (C_{total} - B_{max} - K_D) + \sqrt{(C_{total} - B_{max} - K_D)^2 + 4 \times K_D \times C_{total}} \tag{1}$$

In this equation *C*<sub>unbound</sub> represents the unbound cefazolin concentration, *C*<sub>total</sub> the total cefazolin concentration, *B*<sub>max</sub> the maximum protein binding and *K*<sub>D</sub> the dissociation constant.

Table 1. Clinical characteristics of the patients included in the study

Number of patients	36
Number of samples	119
bBW (g), median (range)	2720 (540–4200)
cBW (g), median (range)	2755 (830–4200)
PNA (days), median (range)	9 (1–30)
GA (weeks), median (range)	37 (24–40)
PMA (weeks), median (range)	38 (25–41)
Albumin (g/L), median (range)	34.5 (28.2–43.7)
Creatinine (mg/dL), median (range)	0.46 (0.26–1.03)
Free fatty acids (mmol/L), median (range)	0.08 (0–0.84)
Indirect bilirubin (mg/dL), median (range)	2.91 (0.1–11.13)
Gender (number of males/number of females)	22/14

For the statistical sub-model, the inter-individual variability was assumed to follow a log-normal distribution. For the intra-individual variability and residual error, we tested proportional, additive and combined error models.

### Covariate analysis

The following covariates were evaluated in the covariate analysis: birth body weight [bBW (g); body weight on the day of birth], current body weight [cBW (g); body weight on the day of blood sampling], PNA (days), gestational age [GA (weeks)], postmenstrual age [PMA (weeks); combination of GA and PNA in weeks], albuminaemia (g/L), creatinaemia (mg/dL), free fatty acids (mmol/L), indirect bilirubin (mg/dL) and gender. Potential covariates were separately implemented in the model using a linear or power equation:

$$P_i = P_p \times \left( \frac{Cov}{Cov_{Median}} \right)^k \quad (2)$$

In this equation  $P_i$  represents the individual parameter estimate of the  $i$ th subject,  $P_p$  equals the population parameter estimate,  $Cov$  is the covariate and  $k$  is the exponent, which was fixed at 1 for a linear function or was estimated for a power function. Covariates were considered statistically significant if the OFV decreased by  $\geq 7.8$  points ( $P < 0.005$ ). The covariate causing the largest reduction in OFV was chosen as a basis to sequentially explore the influence of additional covariates. The choice of the covariate models was further evaluated as discussed under Model development, whereby the results of the model validation were also considered.

### Model validation

The stability of the final pharmacokinetic model was evaluated by a bootstrap analysis, in which the model building dataset was resampled 1000 times in S-Plus version 6.2.1 with NM.SP.interface version 05.03.01. To evaluate the accuracy of the model the normalized prediction distribution error (NPDE) method was used. To perform this analysis the dataset was simulated 1000 times, after which each observed concentration was compared with the simulated concentrations using the NPDE package in R.<sup>29,30</sup>

### Monte Carlo simulations

To evaluate  $T_{>MIC}$ , the CLSI 2012<sup>31</sup> MIC interpretative criteria for susceptibility to cefazolin corresponding to the five bacterial species isolated most frequently from neonatal blood cultures from our department were used. Therefore, all positive blood culture results ( $n=137$ ) from our unit for the period from January to October 2012 were retrospectively collected. Identification of bacterial isolates was done by use of MALDI Biotyper (Bruker Daltonics, Bremen, Germany). *Staphylococcus* species contributed

94.4% of the top five isolates. Consequently, the CLSI MIC interpretative criterion for susceptibility to cefazolin of *Staphylococcus* species (8 mg/L) was used as the target MIC (Table 2).<sup>31</sup>

As effective cefazolin therapy is reported to require at least 60% of  $T_{>MIC}$ , the probability of attaining unbound cefazolin concentrations during 60% of the dosing interval<sup>14</sup>  $>8$  mg/L was evaluated on the basis of Monte Carlo simulations using the final pharmacokinetic model. These Monte Carlo simulations were performed in 1000 individuals to evaluate exposure to cefazolin in (pre)term neonates following the currently used dosing regimen in this study and the dosing regimen proposed by the Dutch Children's Formulary.<sup>15</sup> The covariates identified in the final pharmacokinetic model were sampled from the original dataset taking into account their correlation. Albumin was randomly generated according to the observed distribution in these 36 neonates. For the simulations, cefazolin doses were administered over 30 min every 8 h until 48 h after the first dose. To evaluate the results of the Monte Carlo simulations, four different groups (group 1, PNA  $\leq 7$  days and cBW  $\leq 2000$  g; group 2, PNA  $\leq 7$  days and cBW  $> 2000$  g; group 3, PNA  $> 7$  days and cBW  $\leq 2000$  g; and group 4, PNA  $> 7$  days, cBW  $> 2000$  g) were created. Based on these results, a new model-based dosing regimen was proposed.

## Results

### Patients

The pharmacokinetic analysis was based on 119 plasma concentrations of cefazolin obtained in 36 (pre)term neonates with PNA 1–30 days. Median total and unbound cefazolin plasma concentrations were 101.09 (range 17.44–404.22) and 41.15 (range 5.34–261.38) mg/L, respectively. The median unbound fraction was 0.40 (range 0.14–0.73). Clinical characteristics are presented in Table 1.

### Population pharmacokinetic analysis

#### Structural and statistical sub-model

A one-compartment model was selected as the structural model because a two-compartment model was not superior to a one-compartment model. The final one-compartment pharmacokinetic model, taking into account total and unbound cefazolin concentrations, was parameterized in terms of CL, volume of distribution ( $V$ ),  $B_{max}$  and  $K_D$  (Figure 1). By the determination of  $B_{max}$  and  $K_D$ , unbound cefazolin concentrations could be calculated from total concentrations (Equation 1). Initially, a separate proportional error was estimated for total and unbound cefazolin concentrations. Since these errors were not significantly different

**Table 2.** The five bacterial species isolated most frequently from neonatal blood cultures ( $n=137$ ) in the Leuven neonatal intensive care unit for the period January 2012 to October 2012; corresponding CLSI MIC values are reported

Isolate	Contribution to all positive blood cultures (%)	Contribution to top five isolates (%)	CLSI MIC values (mg/L)		
			susceptible	intermediate	resistant
<i>Staphylococcus epidermidis</i>	51.82	65.74	$\leq 8$	16	$\geq 32$
<i>Staphylococcus hominis</i>	9.49	12.04	$\leq 8$	16	$\geq 32$
<i>Staphylococcus aureus</i>	6.57	8.33	$\leq 8$	16	$\geq 32$
<i>Staphylococcus capitis</i>	6.57	8.33	$\leq 8$	16	$\geq 32$
<i>Escherichia coli</i>	4.38	5.56	$\leq 2$	4	$\geq 8$

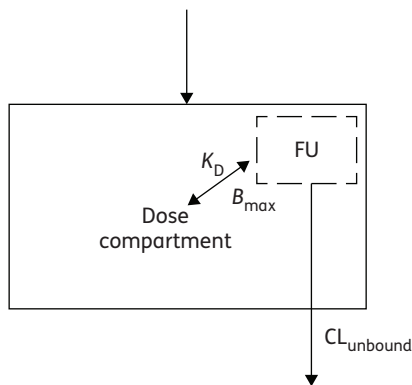
( $P>0.05$ ), the model was simplified by estimating one proportional error for both total and unbound concentrations.

Covariate model

cBW was found to be the most important covariate on  $V$ . Initially, cBW was implemented on  $V$  using a power function with an estimated exponent of 0.94. However, since the 95% CI of this parameter included 1, a linear relationship between cBW and  $V$  was used ( $P>0.05$ ). Implementation of cBW on  $V$  caused a significant drop in OFV of 46 points ( $P<0.005$ ). Although for CL, PMA was

identified as the most important covariate, a combination of the covariates bBW and PNA was preferred over PMA alone. First, both analyses resulted in a comparable improvement of the model (i.e. same reduction in OFV of 32 points,  $P<0.005$ ). Secondly, the combination of bBW and PNA made it possible to distinguish between the antenatal (bBW) and postnatal (PNA) maturation components of cefazolin CL. bBW was implemented on CL using a power function with an estimated exponent of 1.37, while PNA was implemented using a linear function with an estimated slope of 0.496 (Table 3). The model was further improved (reduction in OFV of 12 points,  $P<0.005$ ) by introducing albumin on  $B_{\max}$  using a linear function (Table 3).

The parameter estimates of the simple and final pharmacokinetic models and the values obtained from the bootstrap analysis are provided in Table 3. In Figure 2, the observed versus predicted concentrations are plotted for the total and unbound concentrations, showing that the model adequately described the data. In Figure S1 (available as Supplementary data at JAC Online), the inter-individual variabilities in CL,  $V$  and  $B_{\max}$  are plotted against the relevant covariates for the simple and final pharmacokinetic models. A significant part of the inter-individual variability is explained (Figure S1). This is also reflected in the decrease in the estimates of inter-individual variability when comparing the simple and final pharmacokinetic models, which resulted in a decrease of 50% in the inter-individual variability in  $V$ , 58% in CL and 41% in  $B_{\max}$  (Table 3). In Figure 3 the observed and population predicted bound and unbound cefazolin concentrations are plotted, from which  $B_{\max}$  and the value for the unbound concentration for which the binding was half-maximal ( $K_D$ ) could be derived. Variations in population predicted bound and unbound cefazolin

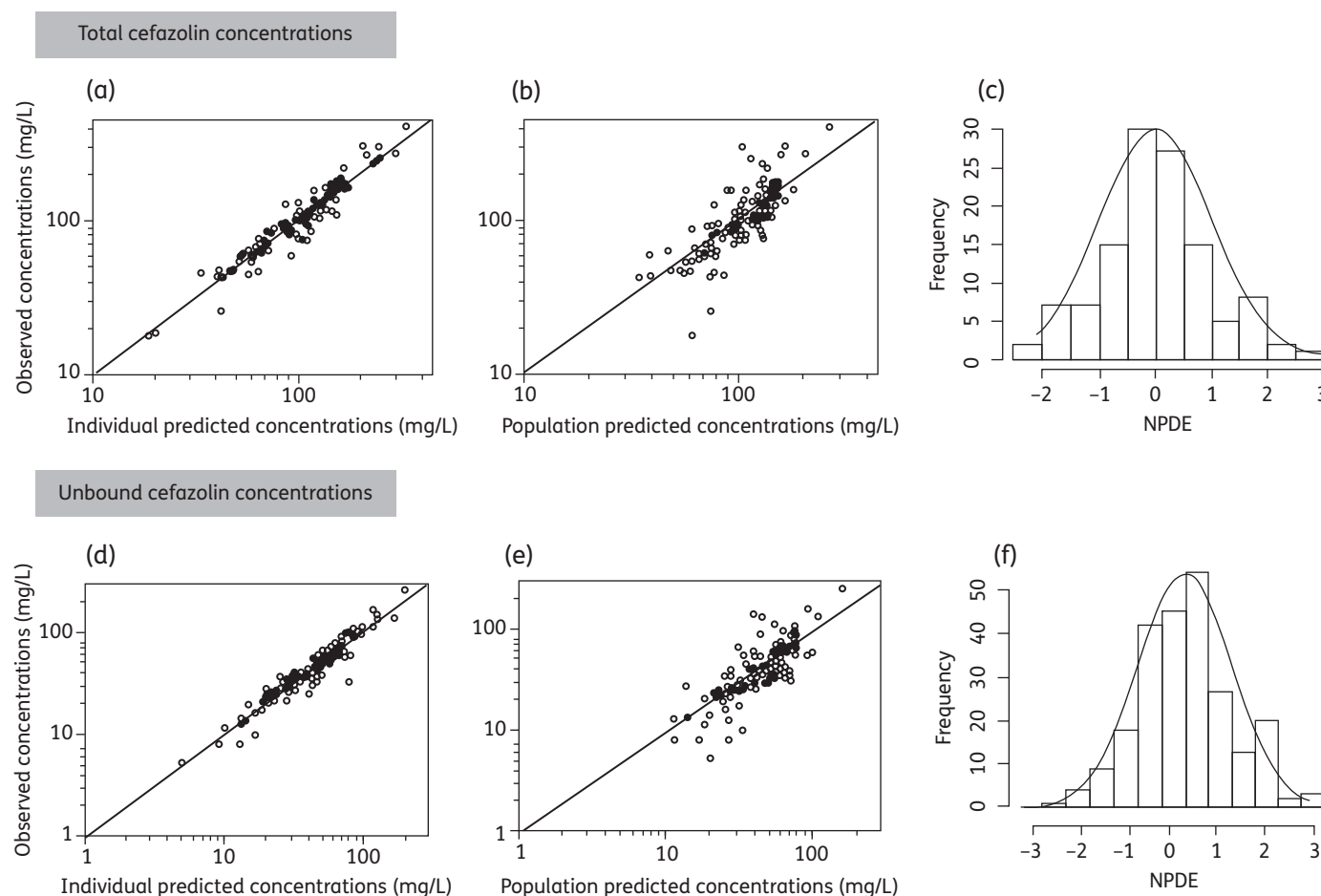


**Figure 1.** Schematic representation of the pharmacokinetic model using both total and unbound concentrations of cefazolin. FU, unbound fraction of cefazolin;  $CL_{\text{unbound}}$ , CL of unbound cefazolin.

**Table 3.** Model-based population pharmacokinetic parameter estimates and values obtained after bootstrap analysis

Parameter	Simple model without covariates: value (CV%)	Final pharmacokinetic covariate model: value (CV%)	Bootstrap final pharmacokinetic model: value (CV%)
Fixed effects			
CL (L/h) = $CL_p$	0.229 (11.7)	—	—
$CL_p$ in $CL = CL_p \times (bBW/median)^m \times [1 + (PNA/median) \times n]$	—	0.185 (12.8)	0.187 (13.3)
$m$	—	1.37 (16.4)	1.41 (17.3)
$n$	—	0.496 (38.5)	0.524 (44.5)
$V$ (L) = $V_p$	0.812 (3.0)	—	—
$V_p$ in $V = V_p \times (cBW/median)$	—	0.863 (3.55)	0.860 (3.63)
$B_{\max}$ (mg/L) = $B_{\max p}$	143 (14.5)	—	—
$B_{\max p}$ in $B_{\max} = B_{\max p} \times (ALB/median)$	—	136 (12.6)	141 (14.5)
$K_D$ (mg/L) = $K_{Dp}$	53.2 (22.9)	46.5 (20.9)	49.5 (24.1)
Inter-individual variability ( $\omega^2$ )			
$\omega^2$ CL	0.535 (33.6)	0.163 (35.1)	0.149 (38.0)
$\omega^2$ $V$	0.14 (29.1)	0.0259 (38.6)	0.0258 (43.2)
$\omega^2$ $B_{\max}$	0.102 (41.0)	0.0367 (54.0)	0.0368 (56.7)
Residual variability ( $\sigma^2$ )			
$\sigma^2$ (proportional)	0.0332 (22.1)	0.0351 (21.5)	0.0342 (22.5)

$CL_p$ , population value of CL for an individual with bBW of 2720 g and PNA of 9 days;  $V_p$ , population value of  $V$  for an individual with a cBW of 2755 g;  $B_{\max p}$ , population value for maximum protein concentration for an individual with an albumin concentration of 34.5 g/L;  $K_{Dp}$ , population value of  $K_D$ ; ALB, concentration of albumin.



**Figure 2.** Observed versus individual predicted concentrations (a and d) and population predicted concentrations (b and e) for total (upper panels) and unbound (lower panels) cefazolin concentrations. The histograms show the distributions of the NPDE method for total (c) and unbound (f) cefazolin concentrations.

concentrations can be explained by differences in cBW, bBW and PNA of the subjects (Figure 3).

The number of binding sites on the albumin molecule was derived from  $B_{\max}$ , which was corrected for the molecular weights of albumin (67 000 g/mol) and cefazolin (454.5 g/mol) (Equation 3) and the median albumin concentration (34.5 g/L) (Equation 4), and was calculated to be 0.6.

$$B_{\max} = 0.136 \text{ g/L} \times \left( \frac{67\,000 \text{ g/mol}}{454.5 \text{ g/mol}} \right) = 20 \text{ g/L} \quad (3)$$

$$\text{Number of binding sites} \left( \frac{20 \text{ g/L}}{34.5 \text{ g/L}} \right) = 0.6 \quad (4)$$

### Model validation

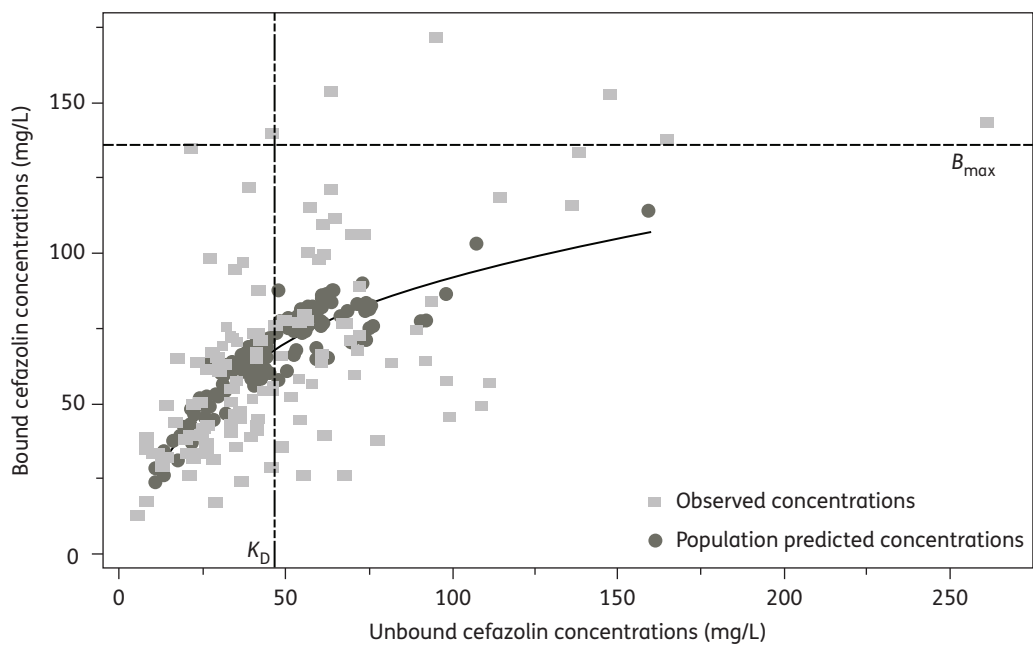
The results of the bootstrap analysis (Table 3) show that the median estimated values based on the resampled dataset were within 10% of the values obtained in the final model. The NPDE histograms follow the normal distribution, indicating the accuracy of the final pharmacokinetic model (Figure 2). Furthermore, no

trend was seen in the NPDE versus time or versus predicted concentrations (figures not shown). The ill-conditioning number (74.6) was far below the critical number of 1000, indicating that the final pharmacokinetic model was not overparameterized. Finally,  $\eta$ -shrinkage, expressed as a percentage, was identified to be 9.8% for CL, 21.2% for V and 30% for  $B_{\max}$ .

### Monte Carlo simulations

Concentration–time profiles following the currently used dosing regimen, the dosing regimen proposed by the Dutch Children's Formulary and the new model-based dosing regimen (Table 4) were predicted based on Monte Carlo simulations using the final pharmacokinetic model (Figure 4). In Figure S2 (available as Supplementary data at JAC Online), box plots illustrate the medians and IQRs (5% to 95%) of the individual predicted concentrations at 60% of the dosing interval after the first dose and after the fourth or sixth dose. This illustrates that <10% of the individual predicted concentrations at 60% of the dosing interval are below an MIC of 8 mg/L. Relatively high cefazolin peak concentrations were reached, particularly in neonates in groups 1, 2 and 3 following the dosing regimen used in the current study and in





**Figure 3.** Relationship between the observed (squares) and model-based predicted (circles) bound and unbound cefazolin concentrations (mg/L) in 36 (pre)term neonates.  $B_{max}$  (protein binding defined as the maximum estimated concentration bound to albumin) and  $K_D$  (defined as the unbound concentration, which corresponds to 50% of the maximum binding capacity) are illustrated.

group 3 following the dosing regimen proposed by the Dutch Children’s Formulary (Figure 4 and Figure S2). Therefore, a new dosing regimen was advised based on the dosing regimen proposed by the Dutch Children’s Formulary, but including a lower dose for group 3 (Table 4). Using this dosing regimen, 0%, 1.2%, 0.7% and 1.0% of the individuals of groups 1, 2, 3 and 4, respectively, would be exposed to concentrations <8 mg/L at 60% of the dosing interval (Figure S2B).

Discussion

Neonatal cefazolin pharmacokinetic data are outdated since they are mainly based on total drug concentrations collected in a limited number of subjects. We aimed to characterize cefazolin pharmacokinetics and its covariates based on both total and unbound drug concentrations. In our study, the median cefazolin CL value (coefficient of variation, %) for a neonate with a bBW of 2720 g and PNA 9 days was 0.185 (12.8) L/h (i.e. 0.068 L/kg/h). This is slightly higher than the earlier reported values of 0.53–1.10 mL/kg/min (i.e. 0.032–0.066 L/kg/h) in 11 neonates receiving 30 mg/kg cefazolin intravenously. Since only the unbound cefazolin is pharmacologically active and total drug concentrations only partially reflect unbound concentrations (Figure 3), we would like to emphasize that unbound concentrations need to be measured instead of using estimated unbound concentrations based on a fixed protein binding percentage. This is of especial relevance in highly protein-bound drugs.

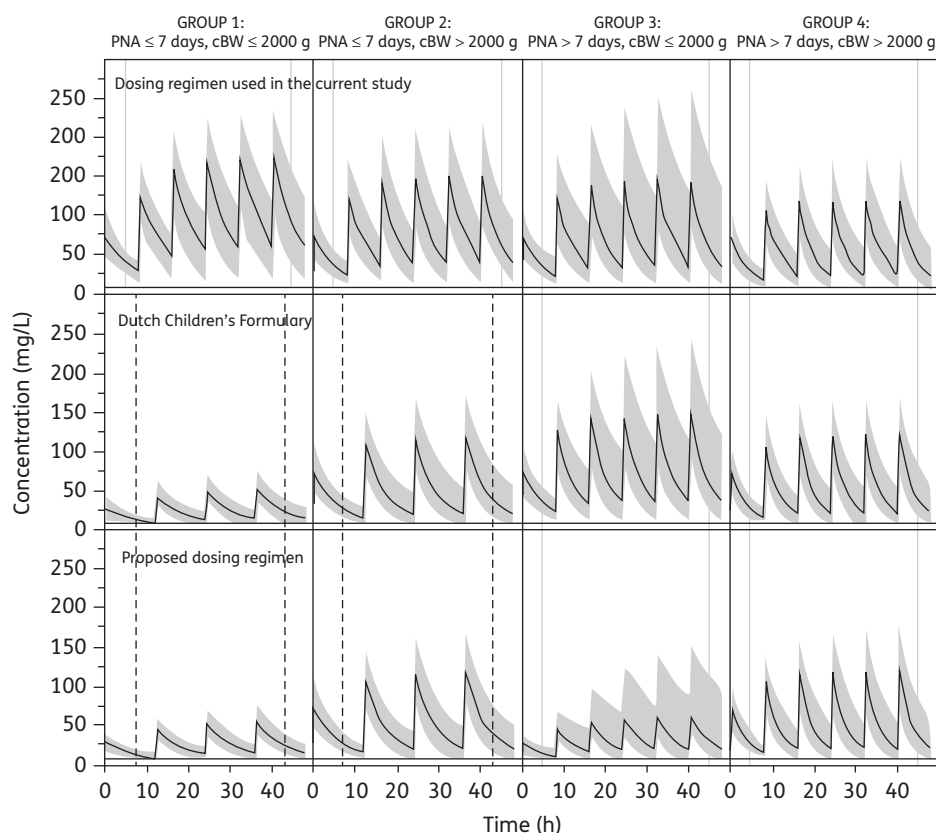
PNA and bBW were the most important covariates of neonatal cefazolin CL. This is in line with expectations, taking into account the elimination of cefazolin by the renal route. Renal CL displays maturation during early life and covariates bBW and PNA can thereby reflect prenatal and postnatal maturation, respectively.<sup>32</sup>

**Table 4.** Dosing recommendations for cefazolin in preterm and term neonates according to dosing regimens used in the current study, the Dutch Children’s Formulary and a new model-based proposed dosing regimen

Guideline	PNA (days)	cBW (g)	Dose (mg/kg)	Interval (h)
Used in the current study	—	—	50	8
Dutch Children’s Formulary	≤7	≤2000	25	12
	≤7	>2000	50	12
	8–28		50	8
Proposed dosing regimen	≤7	≤2000	25	12
	≤7	>2000	50	12
	8–28	≤2000	25	8
	8–28	>2000	50	8

For concentration–time profiles of these dosing regimens for neonates with different clinical characteristics see Figure 4.

Furthermore, age and body weight were earlier documented as CL predictors of other β-lactams in neonates.<sup>33–36</sup> We can only hypothesize about factors affecting the remaining unexplained cefazolin CL variability within the neonatal population. Possibly, maturation of renal tubular activity is a contributing factor. Also for other β-lactams (e.g. amoxicillin, flucloxacillin), the presence of other elimination pathways in addition to glomerular filtration rate, such as tubular secretion or non-renal CL routes, was suggested earlier.<sup>33,37</sup> Since only the unbound drug can be eliminated



**Figure 4.** Concentration–time profiles based on 1000 Monte Carlo simulations using the final pharmacokinetic model following the dosing regimen used in this study (upper row), the dosing regimen proposed by the Dutch Children's Formulary (middle row) and the new model-based proposed dosing regimen (lower row) in four different groups based on cBW and PNA. The black line represents the median of the simulated profiles and the grey area represents the 90% CI of the simulated values. The black horizontal line corresponds to the MIC of 8 mg/L. The full grey vertical lines indicate the time at which 60% of the dosing interval is reached (4.8 and 44.8 h) for a dosing interval of 8 h. The broken vertical lines indicate the time at which 60% of the dosing interval is reached (7.2 and 43.2 h) for a dosing interval of 12 h.

and since compound-specific CL depends on compound-specific protein binding, we want to stress here that the mean  $\pm$  SD protein binding of flucloxacillin ( $74.5 \pm 3.1\%$ ) and in particular amoxicillin ( $11.7 \pm 2.7\%$ ) is lower compared with cefazolin.<sup>34,38</sup> Therefore, results for amoxicillin and flucloxacillin may not be directly applicable to cefazolin.

The number of binding sites for cefazolin on the albumin molecule based on this analysis was calculated to be 0.6 (Equations 3 and 4), which corresponds well to the number of binding sites for cefazolin on albumin previously found in the literature (0.7).<sup>7,39,40</sup>

We documented relatively high cefazolin plasma concentrations based on a cefazolin dosing regimen of 50 mg/kg/8 h, administered to all study patients. This is probably due to the absence of any body weight- and/or age-adapted dosing. Simulation of the dosing regimen proposed by the Dutch Children's Formulary resulted in lower cefazolin concentrations. However, based on Figure 4 and Figure S2, the dose administered to neonates in group 3 when using the Dutch Children's Formulary still needs further reduction. A new body weight- and age-based dosing regimen is suggested, derived from the dosing regimen proposed by the Dutch Children's Formulary, but with a dose reduction for group 3 in order to reach similar exposure in all four groups (Table 4). With this new model-based dosing regimen, the target

of 8 mg/L for 60% of the dosing interval was reached for >90% of the patients (i.e. 100%, 98.8%, 99.3% and 99% of the individuals of groups 1, 2, 3 and 4, respectively).

When compared with the dosing regimen used in this study, a total daily dose reduction of 67%, 33% and 50% for patients in groups 1, 2 and 3, respectively, is proposed, resulting in similar exposure in all groups. The proposed dosing regimen is thus more in line with some of the recommendations presented in Table S1. As a consequence of cefazolin dose reduction, albumin binding places become available for other endogenous (e.g. bilirubin) or exogenous compounds competing for the same albumin binding places. In neonates, frequently showing hyperbilirubinaemia (increased bilirubin production and decreased glucuronidation) and/or receiving multidrug therapies, this is a relevant and population-specific advantage. Recent pharmacokinetic reports of other  $\beta$ -lactam antibiotics commonly used in neonatal intensive care units also suggested dose adaptations compared with previously used regimens. To further illustrate this, a reduction in drug dose and interval for amoxicillin<sup>33</sup> and an increase in initial dose with subsequent dose reduction depending on the microbiological isolate for flucloxacillin<sup>37</sup> were suggested in neonates. This emphasizes the need for population-specific pharmacokinetic studies in neonates. Since study methodologies can differ, a

correct definition of the pharmacokinetic target is required to achieve reliable dosing evaluations in this specific population.<sup>14,41</sup> In general, we have to be aware that total daily dose reduction of an antimicrobial may lead to increased bacterial resistance and ineffectiveness.<sup>42</sup> Prospective validation of the new dosing regimen is therefore necessary, but this was not the intention of the present study.

The strength of our analysis is the measurement of both total and unbound cefazolin concentrations in a relevant neonatal cohort. Additionally, the final pharmacokinetic model can be used to optimize dosing regimens for other pathogens in different settings by changing the target MIC value and/or the  $T_{>MIC}$ . However, there are some limitations. First, the MIC values used were not prospectively determined. Secondly, the success of antibiotic prophylaxis depends not only on selection of the antimicrobial drug and drug dosing, but also on correct, well-timed drug administration and subsequent tissue distribution. Direct measurement of drug concentrations in the surgical site tissues<sup>43,44</sup> may provide additional information to include in pharmacokinetic models, but is very challenging in this population.<sup>45</sup>

We conclude that total and unbound cefazolin concentrations in neonates could be described by a one-compartment pharmacokinetic model that includes saturable protein binding. bBW and PNA were defined as the most important covariates contributing to cefazolin CL variability. A new model-based neonatal cefazolin dosing regimen was proposed; however, prospective validation of this dosing regimen is needed.

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## Transparency declarations

None to declare.

## Supplementary data

Table S1, Figure S1 and Figure S2 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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